California MEDICINE
OFFICIAL JOURNAL OF THE CALIFORNIA MEDICAL ASSOCIATION

Volume 97 DECEMBER 1962 Number 6

Serum Protein Profiles in Coccidioidomycosis

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• **Serum protein analysis is a valuable addition to the present methods for evaluating the status of the individual patient with coccidioidomycosis. The albumin protein and albumin glycoprotein decrease and gamma protein increases in relation to severity of infection. In 40 patients with coccidioidomycosis, changes in individual protein fractions could be significantly correlated with conventional laboratory tests, such as the complement fixation test, erythrocyte sedimentation rate and hematocrit.**

Changes in the alpha$_2$ glycoprotein concentration, the erythrocyte sedimentation rate and the hematocrit value appear to be related to the degree of inflammation, while the changes in the gamma protein and the beta$_2$ glycoprotein appear to be related to the specific antibody response.

COCCIDIOIDOMYCOSIS is usually a relatively benign, self-limited infection. In a few patients, however, the primary pulmonary infection is followed by progressive dissemination that may involve almost any system of the body except the gastrointestinal tract. Because of the ominous prognosis that goes with dissemination, it would be beneficial to have additional objective laboratory tests to evaluate the clinical course and activity of the infection. To explore one possibility, the authors measured the inflammatory process of 40 patients with coccidioidomycosis, using the new electrophoretic and immunochemical techniques now being developed (gamma globulins, alpha$_1$ and alpha$_2$ protein and glycoprotein), and compared the results with those of complement fixation and skin tests with coccidioidin and with the results of conventional laboratory tests.

METHODS

The subjects were 40 consecutive patients admitted to the Kern County General Hospital, Bakersfield, with the diagnosis of coccidioidomycosis. These patients, 30 men and 10 women, represented a fairly typical cross-section of those seen in a county hospital in an endemic area. Their disease, on the basis of its clinical activity, could be divided into four classifications: primary pulmonary infection (nine patients); benign residual coccidioidal lesions of the lungs or pleura (four patients); chronic dissemination (23 patients); and meningitis (four patients, one with additional dissemination elsewhere, and another with a resolving benign lung lesion).

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Submitted July 30, 1962.

In addition, the patients were grouped according to the physician's clinical estimate of the severity of infection into those with "mild" (14 patients), "moderate" (17 patients), and "severe" (nine patients) disease. Placement into one of these groups was made largely on the patient's general appearance and clinical course, without consideration of any laboratory data.

CONVENTIONAL LABORATORY METHODS AND RESULTS

Coccidioidin, a filtrate of liquid cultures of Coccidioides immities, is used for the various tests to measure the status of the disease, such as the skin test, precipitin test and complement fixation test.*

In a "positive" skin test, coccidioidin injected intradermally produces a delayed reaction that is read after 24 and 48 hours. This indicates that the patient has, or has had, an infection with Coccidioides immitis. Almost all patients will have a positive skin reaction after the third week of their infection. Repeated testing does not sensitize the patient's skin to the antigen, nor does it result in a rise in the complement fixation or precipitin titers. The skin sensitivity cannot be passively transferred.[10] Patients with primary infection and erythema nodosum usually show pronounced hypersensitivity upon skin testing.[14] When dissemination occurs, skin reactivity may decrease or disappear. Sensitivity to skin testing does not rule out dissemination, but indicates rather that the individual patient may still possess significant resistance to the infection.[14]

All patients in this study were skin tested. If the result of the first skin test (1:100 strength) was negative, a second test with 1:10 strength was done. Of the 40 patients, 17 had positive reaction with the 1:100 strength, seven with the 1:10 strength, and 16 were negative with both strengths. With increased severity of disease, the skin responses were less distinct. Of the 23 patients with dissemination in this series, four were positive to 1:100 strength, seven were positive to 1:10 strength but not to 1:100, and 12 were negative to both strengths.

The precipitin test is useful in establishing the diagnosis, but it has little prognostic significance. Precipitins usually appear in the serum after the skin test becomes positive, but disappear after three or four months.[11,12] The complement-fixing antibodies, if they subsequently appear, develop more slowly than the precipitins. A rising titer usually indicates the infection is of progressively increasing severity, while regression usually means the infection is waning. A persistently high titer suggests the possibility of chronic dissemination. The complement fixing antibodies do not seem to offer the patient any protection from the infection. Complement fixation tests were performed on all 40 patients in this study. There appeared to be less reactivity to the skin test in those patients having the higher complement fixation titers.

In this series, the authors considered those patients with coccidioidal meningitis as a separate group because they felt that the skin test and the complement fixation titer did not accurately indicate the prognosis when meningitis is present. The complement fixation test may be positive in a low titer and the skin test may be strongly positive, while the opposite may be seen in other forms of dissemination. Isolated meningitis can occur without evident involvement of other systems, as a result of early seeding of the meninges during the acute pulmonary infection.

Other blood tests that are clinically useful in prognosis and objective evaluation of the infection include the leukocyte count, the eosinophil count, the hematocrit value and the erythrocyte sedimentation rate. Leukocytosis roughly indicates the severity of the primary infection, but it has questionable significance with regard to dissemination. Eosinophilia may be associated with erythema nodosum, which is considered a favorable prognostic sign, but on the other hand, high eosinophil counts may be observed in prolonged pulmonary infiltration and may herald dissemination of the infection.[3]

The complement fixation titer, erythrocyte sedimentation rate and mean hematocrit value were all significantly different in the three categories grouped by clinical estimates of severity of infection. The complement fixation titers and erythrocyte sedimentation rates progressively increased with severity of infection, but there was considerable overlap of the values from group to group. There was relatively little difference between the hematocrit values of the patients with "mild" disease and those with "moderate" disease. The lowest hematocrit values were usually associated with severe infection, and the authors found almost no overlap between the hematocrit values of the patients with "severe" infection and those patients with "mild" or "moderate" infection.

C-reactive protein was significantly associated with a progressive increase in severity of infection. Only two of fourteen patients with "mild" disease had positive reaction, while 18 of 26 patients with "moderate" or "severe" infections had positive reaction.

Progressive infection is frequently associated with increased globulin and decreased albumin in the serum. Simple chemical determinations of these proteins have been used empirically by some clinicians as aids in evaluating the severity of the disease. For example, in another study, the serum albumin and globulin levels were determined in 25 cases of dis-

*References 1, 2, 5, 8, 10.

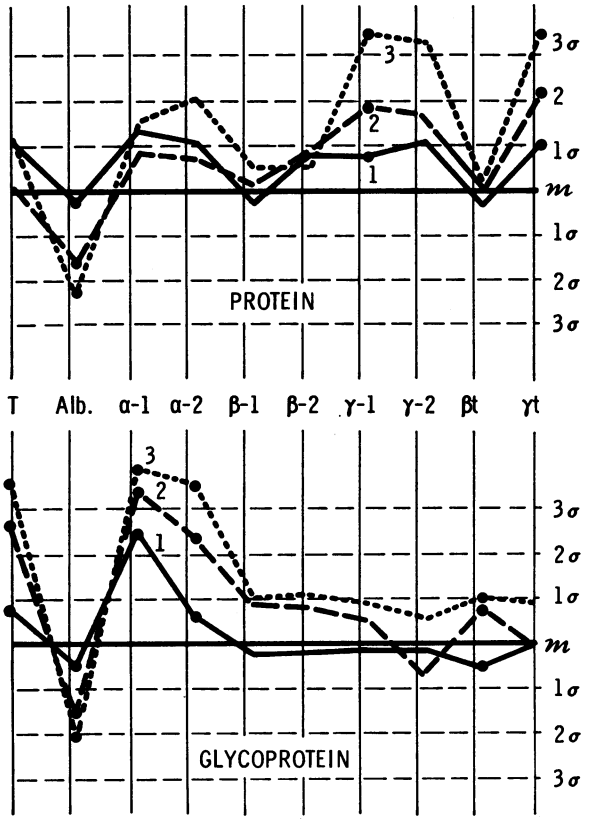

Chart 1.—Mean serum protein profiles (absolute content of patients with relatively "mild" (1), "moderate" (2), and "severe" (3) coccidioidomycosis. Normal mean (m) and standard deviation (σ) indicated by horizontal lines.

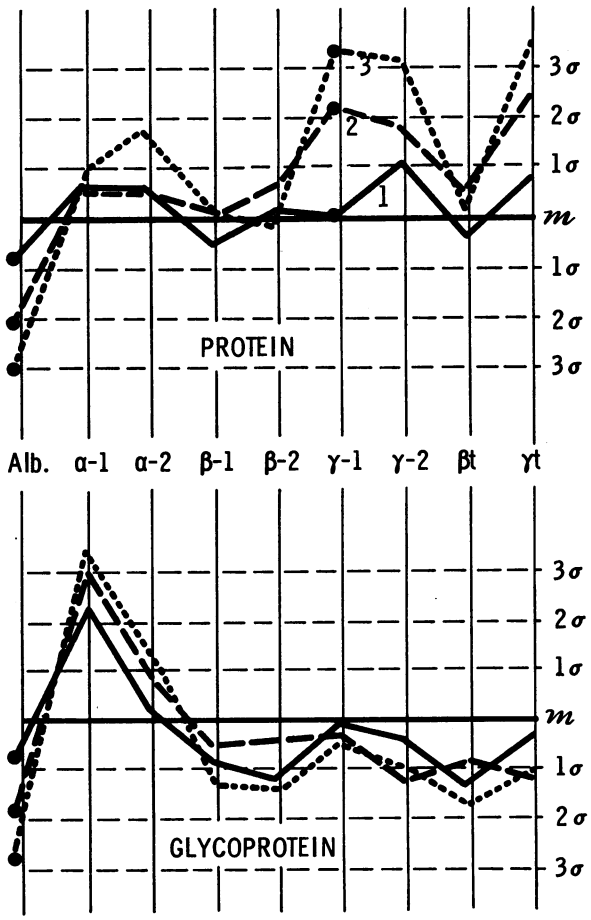

Chart 2.—Mean serum protein profiles (relative per cent) of patients with relatively "mild" (1), "moderate" (2) and "severe" (3) coccidioidomycosis. Normal mean (m) and standard deviation (σ) indicated by horizontal lines.

seminated coccidioidomycosis before and after amphotericin-B therapy.[7] Before treatment, the mean albumin level was 3 gm. per 100 cc. in these patients, and the mean globulin level was 3.9 gm. per 100 cc. After three months of therapy, the mean albumin level had risen to 4 gm. per 100 cc. and the mean globulin level had fallen to 2.8 gm. per 100 cc.

IMMUNOCHEMICAL METHODS

Serum was stored at –10° C. and remained in a thawed state for not more than 24 hours before use in testing. Total serum proteins and glycoproteins were determined by the methods of Weimer and Moshin.[13] Serum electrophoretic patterns were determined with the Spinco system,[9] using the periodic-acid-Schiff stain and the Model RB Analytrol for quantitation. C-reactive protein levels were determined by a quantitative gel-diffusion technique.[4]

The absolute protein and glycoprotein content in each electrophoretic fraction was computed from the product of the total serum concentration and the relative per cent of total migrating in the individual electrophoretic fractions.

IMMUNOCHEMICAL RESULTS AND COMPARISONS

Relationship of Electrophoretic Fractions to Severity of Infection

Serum protein fraction concentration showing significant differences related to progressive increases in severity of infection include the following: the total serum glycoprotein, gamma$_1$ protein, alpha$_1$ glycoprotein, total beta-glycoprotein, albumin protein and albumin glycoprotein. However, there were no significant differences in the electrophoretic patterns of glycoproteins when judged only by their relative per cent distribution, except for a progressive increase in the per cent migrating in the albumin fractions with increasing severity of infection. The electrophoretic patterns of the proteins, however, showed significant differences with respect to the per cent which migrated as gamma$_1$ protein and albumin. With increasing severity of infection, there was a progressive increase in the per cent migrating as gamma$_1$ protein, and a progressive decrease in

the per cent migrating as albumin. (See Charts 1 and 2.)

Correlation of Electrophoretic Fractions with Clinical Tests

Both the absolute and relative concentrations of albumin and gamma$_1$ protein showed significant correlations with the complement fixation titer and the erythrocyte sedimentation rate, and to a lesser degree, with the hematocrit. Both the absolute and relative concentration of the alpha$_2$ fraction showed a significant positive correlation with the sedimentation rate, a negative correlation with the hematocrit value, and no significant correlation with the complement fixation titer.

The total serum glycoproteins showed a significant correlation with the complement fixation titer and the sedimentation rate, but not with the hematocrit values. Most of this increase was due to smaller increases in the alpha$_2$ and beta$_2$ glycoprotein electrophoretic fractions. The hematocrit correlated positively with the absolute and relative concentration of glycoprotein in the albumin electrophoretic fraction, but not with that in the alpha$_2$ fraction.

Relationship of Serum Protein Profiles to the Clinical Type of Disease

There were no characteristic serum protein profiles for the various clinical types of disease. In the four patients with meningitis, there was little alteration of the protein patterns, although there were significant increases in the alpha glycoproteins. Two of these patients, however, also had lesions outside the central nervous system (pulmonary in one, osseous and cutaneous in the other). The four patients with solitary benign pulmonary or pleural lesions showed relatively normal patterns for proteins and glycoproteins, except for increases in alpha$_1$ glycoprotein. Alpha glycoprotein increases are associated with inflammation, and these increases may indicate continued active inflammation in these patients.

DISCUSSION

Of the laboratory tests currently used in the management of patients with coccidioidomycosis, it is generally thought that the complement fixation test offers the best guide to the severity of the infection. Even when this test is readily available, however, there is always need for additional objective and independent laboratory tests to aid in the management of coccidioidomycosis. The serum protein profile, when judiciously interpreted in the light of other clinical and laboratory data, is a valuable additional measure of the severity of the infection.

Unlike the results of complement fixation test, the abnormalities of the serum protein profile have no diagnostic or etiologic specificity. The available clinical evidence indicates that the total serum glycoproteins, particularly those of the alpha$_1$ and alpha$_2$ fractions, primarily reflect inflammation. It is widely thought that the increases in the gamma proteins, and possibly the beta$_2$ proteins, reflect to some extent antibody formation. However, recent studies have demonstrated considerable physico-chemical and immunological heterogeneity of these fractions, and it is likely that, in patients with very high values, only a relatively small portion of this fraction would consist of specific antibodies to Coccidioides, when considered gravimetrically.

The data of this study suggest that variations in the complement fixation titer, erythrocyte sedimentation rate and hematocrit value possibly reflect different patho-physiologic phenomena. The complement fixation titer, presumably a direct measurement of specific antibody response, shows a significant correlation with the absolute and relative concentrations of gamma globulin which is consistent with an antibody response. It is assumed that the negative correlation with albumin is due primarily to the non-specific decrease in this fraction observed in most severe infections or inflammatory diseases associated with the indirect nutritional effects on the serum proteins.

The correlation of the sedimentation rate with the alpha$_2$ protein and glycoprotein fractions is less easily explained. Increases in the sedimentation rate have been attributed to increased fibrinogen, globulins and haptoglobulins. Previous studies of serum protein profiles in other infectious and inflammatory diseases demonstrated a correlation between the alpha$_2$ protein, particularly the alpha$_2$ glycoprotein fraction, and the degree of inflammation.[6] Assuming this relationship exists in coccidioidomycosis, the erythrocyte sedimentation rate would appear to correlate with the degree of inflammation as well as with the antibody response.

The hematocrit was relatively normal in the patients with "mild" or "moderate" infections, with relatively little depression until the infection was "severe." There was also relatively less correlation between the hematocrit and the protein electrophoretic fraction. The relative concentration of the albumin and alpha$_2$ glycoprotein fractions, however, showed a closer correlation.

The physician's clinical estimate of the severity of the infection correlated closely with many of the objective laboratory determinations. The value of the hematocrit in estimating severity of infection is limited somewhat by the lack of a significant decrease until the infection has become extensive. The small overlap of values of patients with "severe" infections and those of patients having "mild" and "moderate" infections indicates a relatively high degree of specificity.

The complement fixation titer showed more variation due to severity of infection than did the erythrocyte sedimentation rate. The increase in these two measurements was also progressive through all three clinical categories, indicating their value in detecting dissemination following the primary infection. The complement fixation titer appears to be the more reliable of the two, because of its specificity and possibly its greater sensitivity. However, it is difficult to perform and is not widely available; and the erythrocyte sedimentation rate will usually be the more useful as a routine test in clinical management, with the complement fixation test used only at monthly or bi-monthly intervals.

Testing for C-reactive protein, which is never found in the serum of healthy patients and is usually associated with clinically significant inflammation, may sometimes be useful in the clinical management of patients with coccidioidomycosis. Most patients who had extensive pulmonary or extrapulmonary disease had C-reactive protein in their serum, while those with mild infections usually did not. The principal limitation of this test is its nonspecificity, in that it may also be positive due to inflammation from causes other than coccidioidomycosis.

The applications of serum protein analysis in the clinical management of coccidioidomycosis requires more extensive evaluation, particularly extended serial studies in individual patients. It compared favorably in this cross-sectional study with other methods for estimating the extent of disease. It is more easily standardized than the complement fixation test, but lacks its diagnostic specificity. The main value of the serum protein analysis will probably be as an additional, independent and objective laboratory test which may be clinically useful in measuring the response of the host to his disease.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the valuable assistance that was given in this study by Charles E. Smith, M.D., University of California School of Public Health, Berkeley; J. Walter Wilson, M.D., University of California School of Medicine, Los Angeles; Marjorie Biddle, Ph.D., Los Angeles County General Hospital; and Norman Levan, M.D., University of Southern California School of Medicine, Los Angeles.

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